Correlation of Nasal Eosinophilia and Response after Nasal Provocation Test in Patients with Nonallergic Rhinitis

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Abstract

Objectives. We aimed to evaluate the relationship between nasal eosinophilia and nasal hyperresponsiveness to allergen extract.

Study Design. Retrospective chart review.

Setting. Academic tertiary rhinologic practice.

Subjects and Methods. We performed allergy tests (skin prick test and multiple allergosorbent test) and nasal cytology for 194 patients with rhinitis symptoms (76 males and 118 females; age, 11-69 years). According to the results, they were classified into 4 groups: group A (allergic rhinitis with eosinophilia, n = 26), group B (allergic rhinitis without eosinophilia, n = 77), group C (nonallergic rhinitis with eosinophilia syndrome, n = 20), and group D (nonallergic rhinitis without eosinophilia, n = 71). We performed a nasal provocation test (NPT) using house dust mite extract and assessed the changes in symptoms and the decrease in acoustic parameters (total nasal volume and minimal cross-sectional area [MCA]).

Results. Patients in group C were more likely to have severe rhinorrhea and sneezing than those in group D (P < .001). After NPT, group C had greater aggravation of nasal obstruction than group D (P < .001). Group C also showed markedly greater MCA changes as compared with group D 15 minutes after the antigen challenge (P = .002). There was significant correlation between the number of eosinophils and an increase in nasal obstruction (r = 0.319, P = .0009), rhinorrhea (r = 0.302, P = .0017), sneezing (r = 0.219, P = .0241), change in the total nasal volume 15 minutes after NPT (r = 0.287, P = .0028), and change in the MCA 15 minutes (r = 0.322, P = .0008) and 30 minutes (r = 0.250, P = .0098) after NPT.

Conclusion. In patients with NAR, nasal eosinophilia is associated with provocative response after NPT. Further research should be performed to elucidate the mechanisms that underlie this phenomenon.

Keywords
rhinitis, nasal provocation test, eosinophilia

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The definition of allergic rhinitis (AR) comprises typical symptoms of rhinitis, such as nasal obstruction, rhinorrhea, sneezing, and itching and a positive result to a suspected causative antigen in a skin prick test (SPT).1 Clinical features of patients with nonallergic rhinitis (NAR) are quite similar to those of patients with AR, but their SPT results are negative. Previously, all patients with a negative SPT result were merely diagnosed with NAR or noninfectious NAR.2 However, attempts have been made to further subcategorize NAR.3 Therefore, methods for diagnosing these subcategories have also been developed.

NAR with eosinophilia syndrome (NARES) can be diagnosed when the number of eosinophils is elevated in patients with typical nasal symptoms and negative SPT results.4 We can perform a nasal smear and measure the fraction of eosinophils in nasal secretion to aid in the diagnosis of NARES. This method is relatively noninvasive and can quickly evaluate nasal eosinophilia.3 Therefore, outpatient clinics widely use the nasal smear technique in clinical practice.

Local AR (LAR), however, is defined as a state of intrinsic Th2 allergic inflammation in the nasal cavity without systemic allergic sensitization.5,6 Therefore, to diagnose LAR and evaluate local allergic reactions in the nasal cavity, we should perform a nasal provocation test (NPT) in addition to an SPT.7 The NPT evaluates deterioration of nasal symptoms and changes in nasal cross-sectional area/volume after a causative antigen is sprayed into the nasal cavity. This is thought to cause allergic reactions.8,9

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Since we use different methods for the diagnosis of NARES (nasal smear) and LAR (NPT), these 2 diagnoses are not entirely exclusive but have overlapping aspects. For example, if nasal smear shows eosinophilia and if NPT shows a positive result, the patient could be diagnosed with both NARES and LAR. Therefore, it is necessary to evaluate the relationship between nasal eosinophilia and NPT results. In fact, it was reported that nasal eosinophilia helps to predict bronchial hyperresponsiveness.10 Similarly, if nasal eosinophilia can be used to help predict the reactivity of the nasal cavity to an antigen, it will be of great use in clinical practice. However, research on this is still very insufficient.

We classified patients with rhinitis symptoms into 4 groups according to the results of an SPT and nasal eosinophilia (AR with or without nasal eosinophilia, NARES, and NAR without eosinophilia). Then we compared clinical symptoms among these groups. We also investigated the association between nasal eosinophilia and the NPT by performing an NPT and comparing the changes in symptoms and acoustic parameters (volume and dimension) among all patients.

Materials and Methods

Subjects
This study included 194 patients (76 males and 118 females, 11-69 years old, mean ± standard deviation [SD] age = 30.8 ± 13.8 years) who visited our hospital’s ear, nose, and throat department for at least 1 year of persistent rhinitis (nasal obstruction, rhinorrhea, sneezing, and postnasal drip). We excluded those who had used antiallergic treatment drugs (eg, antihistamines and intranasal/systemic steroids) for over a month, those with unstable systemic cardiovascular or pulmonary disorders (including asthma), female patients who were pregnant or in the lactation period, patients who had gotten nasal surgery (eg, endoscopic sinus surgery and/or septoplasty) within the last 3 months, those with chronic rhinosinusitis and/or nasal polyposis (confirmed by rigid rhinoscopy and/or paranasal radiography), and current/past smokers. We received approval from the Institutional Review Board Committee on Studies Involving Human Beings (INHAUH 2017-12-017-001), and we conducted a retrospective chart review of the patients.

Allergy Tests
We performed SPTs and multiple allergosorbent tests (MASTs) on all patients for >40 antigens, including those from house dust mites, fungus, tree or weed pollen, pets (cats or dogs), and cockroaches. We defined patients with AR as those with strong positive results to house dust mite (for SPT, a wheal/erythema diameter ≥6 mm [the same as histamine]; for the MAST, score ≥3) and typical symptoms of rhinitis when exposed to the corresponding antigen. We also classified patients as having NAR when the results for any antigen on SPT or MAST test were entirely negative.

Nasal Cytology and Classification of Patients
We performed a cotton nasal swab for cytology in both nostrils of patients, according to previous well-established methodologies.3 We dilated the nostrils of patients with a nasal speculum and collected samples of nasal secretion in the middle of the inferior turbinate. We rubbed the swab several times on a glass slide. After the glass slide was dried and fixed at room temperature, the sample was stained with the May-Grunwald Giemsa method. Experienced examiners who were blind to the purpose of this study randomly selected 10 microscopic fields and measured the number of cells with light microscopy. We diagnosed patients with nasal eosinophilia when >20% of eosinophils were in the nasal cytology.11 Patients without nasal eosinophils were those with 0% eosinophils in their nasal secretion samples.

According to the results of allergy tests and nasal cytology, we classified patients into 4 groups: group A (AR with eosinophilia, n = 26), group B (AR without eosinophilia, n = 77), group C (NARES, n = 20), and group D (NAR without eosinophilia, n = 71). The demographic data of patients are summarized in Table 1.

Baseline Symptoms and Severity in Patients
At the time of their initial visit, all patients were asked to complete a visual analog scale (VAS) questionnaire indicating their subjective degree of discomfort for each nasal symptom (nasal obstruction, rhinorrhea, sneezing, and itching sensation). The VAS ranged from 0 (not at all inconvenient) to 10 (very uncomfortable).

We also assessed severity among patients according to the ARIA 2008 classification (Allergic Rhinitis and Its Impact on Asthma)12 by asking whether they experienced symptoms for >4 days per week and/or for 1 month in a year and by asking the extent of the impact of symptoms on their quality of life.

NPT and Acoustic Rhinometry with House Dust Mite Antigen
Methods for carrying out the NPT were described in detail in our previous work.13,14 To summarize, patients were first acclimatized by waiting for 15 minutes in a room with a constant room temperature (22°C) and relative humidity (50%). During waiting, they completed a VAS about the severity of nose symptoms as baseline data before any nasal challenge. We measured total nasal volume (TNV; the sum of the cross-sectional area from the nostril to 7 cm deep) and minimal cross-sectional area (MCA; the smallest cross-sectional area of the nasal cavity) with acoustic rhinometry (E. Benson Hood Laboratories, Allergopharma, Hamburg, Germany) before any intranasal challenge (baseline data).

First, to evaluate nonspecific hyperreactivity, we sprayed about 50 μL of normal saline into both nasal cavities with a pump sprayer. After 5 minutes, we repeatedly measured TNV and MCA with acoustic rhinometry.
Table 1. Demographic Data of Patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>Male</th>
<th>Female</th>
<th>Age, yrs, Mean ± SD</th>
<th>ARIA Classification, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>16</td>
<td>10</td>
<td>26.4 ± 9.8</td>
<td>Intermittent: 4, Persistent: 22, Mild: 5, Moderate to Severe: 21</td>
</tr>
<tr>
<td>B</td>
<td>49</td>
<td>28</td>
<td>25.6 ± 10.5</td>
<td>Intermittent: 20, Persistent: 57, Mild: 20, Moderate to Severe: 57</td>
</tr>
<tr>
<td>C</td>
<td>13</td>
<td>7</td>
<td>45.8 ± 10.3</td>
<td>Intermittent: 5, Persistent: 15, Mild: 6, Moderate to Severe: 14</td>
</tr>
<tr>
<td>D</td>
<td>45</td>
<td>26</td>
<td>35.9 ± 15.1</td>
<td>Intermittent: 13, Persistent: 58, Mild: 16, Moderate to Severe: 55</td>
</tr>
</tbody>
</table>

Abbreviation: ARIA, Allergic Rhinitis and Its Impact on Asthma.

*Group A, allergic rhinitis with eosinophilia; group B, allergic rhinitis without eosinophilia; group C, nonallergic rhinitis with eosinophilia syndrome; group D, nonallergic rhinitis without eosinophilia.

After waiting for an additional 10 minutes for the effect of saline to disappear, we performed an intranasal challenge with *Dermatophagoides pteronyssinus* (DP) allergen extract (50,000 standardized biological units per milliliter, diluted to 1:10 with 0.9% normal saline, approximately 50 μL; Allergopharma) into both nasal passages of patients. Fifteen and 30 minutes after the DP challenge, we readministered the VAS and repeated acoustic rhinometry to measure changes in each parameter. Changes in nasal symptoms were calculated as (VAS score after DP provocation) − (VAS score before DP provocation). Changes in TNV or MCA were calculated as [(TNV or MCA baseline value − TNV or MCA after provocation) / TNV or MCA baseline value] × 100 (%).

Statistical Analysis

We used Prism 5 (GraphPad Software, Inc, La Jolla, California) and SPSS 18.0 (IBM, Chicago, Illinois) for statistical analysis. Kruskal-Wallis with post hoc Dunn test was used to determine whether differences between groups were statistically significant. Since data do not follow a normal distribution, we used a nonparametric method. We used Spearman’s method to examine correlations between nasal eosinophilia and changes in symptoms/acoustic parameters after NPT. Data were presented as median ± interquartile range, and *P* values <.05 were considered statistically significant.

Results

Comparing nasal symptoms among patients at their initial visit, we found no statistically significant difference between group A (AR with eosinophilia) and group B (AR without eosinophilia, *P* > .05). However, patients in group C (NARES) were more likely to have more severe rhinorrhea and sneezing symptoms than group D patients (NAR without eosinophilia, *P* <.001; Figure 1).

When saline was sprayed into the nasal passages of patients, we found no significant differences in changes of TNV and MCA (*P* > .05 for both; Figure 2).

Thirty minutes after DP antigen intranasal challenge, patients in group C had greater aggravation of nasal obstruction than group D (*P* < .001; Figure 3).

When changes in TNV and MCA were compared after exposure to DP allergen extract, groups A and B showed no statistically significant difference (*P* > .05). However, group C showed markedly greater MCA changes than group D at 15 minutes after antigen challenge (*P* = .002; Figure 4).

For patients in groups C and D, we used Spearman’s methods to examine correlations between nasal eosinophilia and changes in symptoms and acoustic parameters after NPT. As a result, we found a statistically significant correlations between nasal eosinophilia and changes in nasal obstruction, rhinorrhea, and sneezing; changes in TNV 15 minutes after NPT; and changes in MCA 15 and 30 minutes after NPT (*P* <.05; Table 2).

Discussion

In our study, the severity of baseline symptoms was not significantly different between group A (AR with eosinophilia) and group B (AR without eosinophilia)—perhaps because immunoglobulin E, as secreted from mast cells, plays a significant role in the pathophysiology of AR. However, group C (NARES) showed a statistically significant tendency for rhinorrhea and sneezing to be more severe than in group D (NAR without eosinophilia). Also, nasal obstruction and itching symptoms were more severe in group C than D (although not statistically significant). Moreover, Total Nasal Symptom Score was significantly higher in group C than in group D. Therefore, nasal eosinophilia may play a more significant role in worsening local nasal symptoms in patients with NAR without immunoglobulin E–mediated allergy. In our previous study, we found no difference in baseline symptoms between patients with AR and those with NAR. However, our previous study differs from the current study because patients with nasal eosinophilia were excluded in it. According to the results of our literature review, this study is the first to investigate differences in clinical features according to the presence of nasal eosinophilia in patients with AR and NAR.

Compared with group D, group C showed statistically significant worsening of nasal obstruction. However, there was no significant difference in changes in rhinorrhea, sneezing, and itching between groups C and D (in both groups of NAR, these symptoms were not significantly aggravated after DP challenge). In our previous study, patients with NAR did not experience significantly
worsened symptoms of rhinorrhea, sneezing, and itching as compared with patients with AR.\textsuperscript{9} In our study, groups C and D showed almost no decrease in rhinorrhea, sneezing, and itching symptoms after DP antigen challenge. Therefore, the clinical features of patients with AR and those with NAR after NPT are very different. In this study, the provocation protocol was to measure the change in symptoms 30 minutes after the DP challenge. Since this is a measurement of relatively early response, it can be assumed that the role of eosinophils, which is mainly involved in late response, is relatively small. Research that can prove these hypotheses in the future should be supported.

We assessed nonspecific hyperreactivity in patients with AR and NAR by assessing the response of the nasal cavity (ie, the change in acoustic parameters) after saline challenge in the nasal cavity to evaluate nonspecific nasal hyperreactivity. Values are presented as median ± interquartile range. Group A, allergic rhinitis with eosinophilia; group B, allergic rhinitis without eosinophilia; group C, nonallergic rhinitis with eosinophilia syndrome; group D, nonallergic rhinitis without eosinophilia.

**Figure 1.** Nasal symptoms of patients at the initial visit as measured by visual analog scale. TNSS, Total Nasal Symptom Score. Values are presented as median ± interquartile range. **\(P < .01\) (vs group A). ***\(P < .001\) (vs group A). Group A, allergic rhinitis with eosinophilia; group B, allergic rhinitis without eosinophilia; group C, nonallergic rhinitis with eosinophilia syndrome; group D, nonallergic rhinitis without eosinophilia.

**Figure 2.** Changes in TNV (total nasal volume) and MCA (minimal cross-sectional area) after saline challenge in the nasal cavity to evaluate nonspecific nasal hyperreactivity. Values are presented as median ± interquartile range. Group A, allergic rhinitis with eosinophilia; group B, allergic rhinitis without eosinophilia; group C, nonallergic rhinitis with eosinophilia syndrome; group D, nonallergic rhinitis without eosinophilia.
nonspecific, nonallergenic stimuli, such as sudden changes in temperature, cold air, perfume, and cigarette smell in patients who have been suffering from rhinitis for a long time. Therefore, we could suggest that nonspecific hyperreactivity is independent of allergy and nasal eosinophilia.

It is also worth noting that there was no significant difference between groups A and C for changes in nasal obstruction after antigen challenge. However, in group A, symptoms of rhinorrhea, sneezing, and itching increased after antigen challenge, but these symptoms did not deteriorate significantly in group C. Therefore, it would be helpful to use symptom change, such as rhinorrhea, sneezing, and itching after NPT, to distinguish AR from NARES.

Changes in TNV and MCA were measured and calculated with acoustic rhinometry before and after the DP challenge. As a result, there were no statistically significant differences between group A and group B in changes in TNV and MCA. This result is in agreement with our finding that there was no statistically significant difference between these 2 groups in changes in nasal obstruction symptoms after NPT. Therefore, we can infer that nasal eosinophilia does not significantly affect the cross-sectional area and volume of the nasal cavity and, thus, the deterioration of nasal congestion of patients with AR.

In the NAR groups, group C showed significantly higher MCA changes than group D 15 minutes after DP intranasal challenge. This result is also in agreement with the results that nasal congestion was significantly worsened after antigen challenge in group C versus group D. Therefore, it would be better that we check nasal eosinophilia, changes in nasal obstruction symptoms, and TNV/MCA after antigen challenge more carefully among patients with NAR.

Previous studies showed that the number of eosinophils increases after antigen challenge in patients with AR. However, to date, there has been no report that nasal eosinophilia causes a more significant NPT response. Therefore, our study has differentiation and significance from other papers in that it can predict the degree of NPT response of patients with nasal eosinophilia. It would have been a more interesting study if the nasal smear was performed before and after NPT to measure eosinophil count and to find any correlation between the degree of eosinophil increase and NPT response.

Based on these results, we conducted a Spearman’s analysis to identify the association between nasal eosinophilia and (1) the degree of deterioration of each nasal symptom and (2) the degree of change in acoustic parameter after NPT among patients with NAR. As a result, there was a statistically significant correlation between nasal eosinophilia and changes in nasal symptoms and acoustic parameters after NPT. Therefore, nasal eosinophilia, especially in
patients with NAR, is associated with the provocative response after NPT. More extensive research with a larger study population is needed to better support this conclusion.

How can we explain the association between nasal eosinophilia and the NPT-positive result? When the decisive criterion for the NPT was defined as a decrease from the baseline by >30% of MCA, 8 of 20 (40.0%) patients in group C and 10 of 71 (14.1%) in group D satisfied the criterion. In other words, in the NARES group, 2.8-times more LAR was observed than in the NAR group without eosinophilia. Therefore, we should keep in mind the possibility that LAR, due to local Th2 allergic inflammation, may also be present in patients with NARES and nasal eosinophilia.

As is well known, eosinophilia is mainly associated with late-phase response in nasal allergic response. Therefore, the results would probably have been more interesting if we had measured changes in symptoms and acoustic parameters 4 to 6 hours after NPT. A limitation of this study is that it evaluated only responses in the early phase. However, it is almost impossible to evaluate late-phase response after NPT when we perform clinical outpatient surveys because of time and space constraints. Therefore, we think that it is more useful to evaluate early-phase response clinically.

One of the weaknesses of our study is that nasal swab eosinophilia is not precisely comparable to nasal mucosal eosinophilia. Moreover, in the same person, eosinophil counts can be measured differently in nasal swabs due to

**Table 2.** Spearman’s Analysis to Reveal Correlation between Eosinophils in Nasal Secretion and Changes in Symptoms and Acoustic Parameters after NPT.

<table>
<thead>
<tr>
<th>Dependent Variablea: Change in  . . .</th>
<th>Spearman’s r</th>
<th>P Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal obstruction symptom</td>
<td>0.319</td>
<td>.0009</td>
</tr>
<tr>
<td>Rhinorrhea symptom</td>
<td>0.302</td>
<td>.0017</td>
</tr>
<tr>
<td>Sneezing symptom</td>
<td>0.219</td>
<td>.0241</td>
</tr>
<tr>
<td>Nasal itching symptom</td>
<td>0.087</td>
<td>.3746</td>
</tr>
<tr>
<td>15 min after NPT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNV</td>
<td>0.287</td>
<td>.0028</td>
</tr>
<tr>
<td>MCA</td>
<td>0.322</td>
<td>.0008</td>
</tr>
<tr>
<td>30 min after NPT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNV</td>
<td>0.118</td>
<td>.2268</td>
</tr>
<tr>
<td>MCA</td>
<td>0.250</td>
<td>.0098</td>
</tr>
</tbody>
</table>

Abbreviations: MCA, minimal cross-sectional area; NPT, nasal provocation test; TNV, total nasal volume.

aIndependent variable: number of eosinophils in nasal secretion (%).

bBold indicates statistical significance, P < .05.
various factors. Therefore, additional studies, such as repeated nasal swab eosinophilia, are essential for the same patient to make the results of this study more credible.

In conclusion, in patients with NAR, nasal eosinophilia is associated with provocative response after NPT. Further research should be performed to elucidate the mechanisms that underlie this phenomenon.

Author Contributions
Ki-Ik Park, main writer (main role for the first writing of whole script), data analysis; Tae Young Jang, main writer, data acquisition and interpretation; Seung-Chan Yang, secondary writer and English interpretation, data analysis, and article revision; Hyung Sun Hong, English editing and statistical analysis, figure work; Young Hyo Kim, study design, responsibility for the paper, revision of the paper.

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References